THE COAGULATION OF CONDENSED MILK.

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Much has been written about the sliminess of milk and of the bacteria which bring about the change. But the literature respecting a similar change in condensed milk is so scanty that I have not been able to find any publication dealing with the subject. It may be that the matter has never been treated, and yet for reasons that will be seen later, the trouble can hardly be unknown to the manufacturers of condensed milk.

The specimen of coagulated or "jellified" milk which I received had been made some six months previously, and when opened, the milk was seen as a stiff, rather dark-tinted jelly of the consistency of stiff starch-paste. Upon vigorously stirring the milk, it became thin but returned to its stiff consistency upon standing.

The microscopical examination of the jelly showed amongst the crystals of lactose, a number of clusters of needle-shaped crystals. These were also seen in normal condensed milk, but were not so numerous. The clusters were not affected by dilute alkali but dissolved in dilute acid, leaving a nucleus or residuum of microbic cells. A small quantity of these crystals was obtained by centrifugalising the diluted milk, and dissolved in nitric acid and tested for phosphoric acid, with negative results.

It was difficult to say whether the microbes were sarcinæ or staphylococci; they were large-sized and stained deeply, the stain being retained by the Gram method. They occurred in pairs and in groups, each individual measuring $1{\cdot}5\mu$. Upon cultivation they grew as small cocci, 1μ in diameter.

The formation of the jelly does not proceed uniformly throughout the milk, but begins at a number of points. These appear as small blobs of the size of millet seed. The thickening then

proceeds radially, and finally the enlarged blobs or lumps fuse together into a solid jelly. This occurs when the milk is at rest. When agitated from day to day, as was done in the experimental work which will be mentioned later, the thickening begins at the surface-margin and proceeds inwards and downwards.

The manufacturer considered that the trouble was caused by a fault in the condensing plant, which, he considered, permitted the entry of water carrying salts of lime and magnesia in solution, and this appeared to be a feasible explanation in view of some experiments that were made by the Department of Agriculture of Victoria. Briefly, these experiments consisted in adding small quantities of lime salts ($=0{\cdot}02\%$ of $CaCO_3$) and of magnesia salts, and incubating the milk thus treated. It was concluded that small quantities of lime and magnesia salts bring about a thickening or coagulation of condensed milk, and in this connection it was noted that saccharated lime or viscogen is used commercially to thicken cream.

The action of the small quantities of calcium carbonate was so unexpected, that I repeated the experiment with specimens of condensed milk from the same firm. These specimens were described as showing no tendency to coagulate. A control test was made at the same time and treated in the same way with the exception that the calcium and magnesium salts were not added. Both tests were retained in the flasks in which the milks had been agitated for two hours at 54° previous to incubation at 37°. The contents of the flask with the salts showed signs of thickening on the fifth day, when a few nodules were visible at the surface margin, and it appeared as if the salts had induced the formation of the viscosity. Then the milk began to thicken from the margin of the surface inwards. At this time, however, it was noted that the check-test had also begun to thicken. The coagulation proceeded in both tests until in about a month the contents of each flask had become a jelly. The only difference between the two was that the test with the salts had a start of two or three days over the control. Both of these milks contained the coccus which had been in the original condensed milk.

A tin of milk which had been made after the supposed fault in the condensing apparatus had been remedied and which was said to be capable of standing prolonged incubation without change, was placed in the incubator for ten days. Upon opening the tin and examining the milk, a considerable number of small blobs of jelly were found distributed throughout the milk. These blobs contained the micrococcus. It is therefore evident that the infection and subsequent alteration of the milk had little if anything to do with the entry of water containing calcium and magnesium salts in solution.

The micrococcus was obtained in pure culture by plate-cultivation and its bacterioscopic characters were noted. With the pure culture several experiments were made. In one of these, portions of approximately 25 c.c. of Nestlé's condensed milk, which keeps well in this climate, were transferred into a series of sterile flasks. Two of these had 0·0025 grm. of chalk added, and all were heated in a water-bath at 60° for an hour. The flasks were then infected, covered with rubber-caps, and placed in an incubator at 37°.

	11 days.	19 days at 37°.
milk (control)	no change	no change
,, + coccus	thickened	very much thickened
,, ,, + *B. acidi lactici*	,,	,, ,,
,, + chalk (control)	no change	no change
,, ,, + coccus	very much thickened	coagulated

The experiment clearly shows that the coagulation of the milk is brought about by the micrococcus and that the alteration can be assisted by small quantities of calcium carbonate.

A repetition of the experiment, using Nestlé's milk which had been heated for six hours in its tin at 60,° was made.

	14 days.	27 days at 37°.
milk (control)	no change	no change
,, + coccus	much thickened	very much thickened
,, ,, ...	,, ,,	,, ,,
,, + chalk (control)	no change	no change
,, ,, + coccus	much thickened	very much thickene

A tin of Nestlé's milk was heated at 60° for two hours, then the lid was punctured and the milk infected with a blob from a coagulating milk. The small orifice was sealed with paraffin and the tin was incubated for a month at 37°. Upon opening the tin it was found that the milk in the neighbourhood of the orifice had become coagulated. The infecting blob had remained at the point of infection and had not mixed with the bulk of the milk.

Another experiment was made with a tin of pasteurised Nestlé's milk, but in this case the milk was infected by means of small capillary tubes containing pure cultures of the micrococcus; the thin tubes were pushed right into the milk and were then broken off. The small hole in the tin was then sealed with paraffin as before. Upon opening the tin a month later, the contents were found to be very stiff and lumpy, a signal evidence of the action of the micrococcus. A control-tin of milk which had been opened and sealed at the same time was unaltered.

It is clear from these experiments that it is the micrococcus which is responsible for the coagulation of the condensed milk.

The causative micrococcus is probably by no means rare, and, as I have separated it from a sample of Nestlé's milk which is prepared in Switzerland or Norway, it would appear to be of universal occurrence. The Nestlé's coccus was identical morphologically and culturally, and it produced the same characteristic coagulation and lumpiness in test-flasks.

Since the coccus is found in milks which keep perfectly, there must be some condition which is necessary in order that the change may occur. The most feasible is the presence of a quantity of air in the tins. The coccus is aërobic, and in the experimental flasks the thickening begins where the film of milk is thinnest and most completely aërated, that is, at the margin of the surface. In the tins in which the affected milk was contained, there was a considerable space filled with air, while in the tins of Nestlé's milk the air-spaces were very small. In the latter case it is possible that the gas is inert, and, if so, the condition for the growth of the coccus would be so unfavourable that no alteration would occur.

On the other hand, air does not appear to be an absolute necessity. This was shown in an experiment in which the influence of air was tested. A series of portions of Nestlé's condensed milk was put into wide tubes, covered with vaseline, and heated for an hour at 60°. These were infected through the small central hole that formed on cooling the tubes. Then a thicker layer of melted vaseline was superposed. The tubes were incubated at 37° for two months. Upon removing the vaseline and examining the contents, it was found that the milks were thicker than when they were put into the tubes. Control tests with and without chalk were similar, while others sown with the coccus with and without chalk showed swollen masses near the vaseline where the milk had been infected. The coccus in the tube with the chalk produced the larger mass of coagulated milk. This experiment makes it appear that the presence of air is not a necessary condition for the coagulation of the milk, and that the addition of lime-salt, such as the carbonate, undoubtedly accelerates the thickening.

With regard to the nature of the substance that forms the jelly, it may be a slime or gum derived from the saccharose or lactose through the biochemical activity of the microbe. On the other hand it may be altered casein. To elucidate this question, many experiments were made in an endeavour to induce the coccus to form slime on artificial media, but all were fruitless, and I was driven to the alternative that the microbe simply alters the casein. There is some reason for the belief that the coagulation is an alteration of the casein. The microbe produces a considerable amount of acid in media containing lactose such as milk or lactose nutrient agar. Milk is coagulated and the acidity of the whey is so pronounced as to make it appear evident that the coagulation is brought about by the acid and not by a production of bacterial rennin-like bodies. Experimental plates of milk-agar and litmus-milk-agar, when seeded with a giant colony of the coccus, showed an amœboid growth upon the surface of the agar; but underneath and for some distance around the amœboid processes the milky medium was opaque while the other parts of the medium were translucent.

The supposition that the coagulation was entirely an acid coagulation was, however, shown to be wrong by the following experiment. Infected milk, with and without the addition of chalk, was poured into separate Petri dishes which were incubated at 22° for three days, when they were transferred to the incubator at 37°. Four hours later, the chalk-test had coagulated while the other had partly coagulated. The reaction of the coagulated milk with the chalk was neutral, while the partly coagulated milk was acid. The neutral reaction of the coagulated milk with chalk shows that the thickening was not caused by the formation of acid, but resulted from the action of an enzyme secreted by the micrococcus.

The coagulated milk is more acid than uncoagulated milk of the same maker; for example, a 20% solution upon being tested showed an acidity to phenolphthalein equal to 42 c.c. of $\frac{N}{10}$ per 100 grm., while an uncoagulated milk equalled 24 c.c. Much the same fact was shown in an experiment in which dilute acid was added to 50 c.c. of the 20% solution of the milk in a bottle. The volume of $\frac{N}{10}$ acid required to produce incipient coagulation, visible as minute specks upon the side of the bottle after shaking, was noted. The coagulated milk required 9·6 c.c., and the uncoagulated 11·1 c.c. These experiments should be considered in conjunction with the fact that the tins had been opened, and the milks exposed to the air for some time, the coagulated milk for 7 days, the uncoagulated for a month.

Experiments were made to determine the lethal temperature, but at the time this was done, the micrococcus had apparently deteriorated, as an exposure in milk at 63° for 10 minutes sufficed to kill it. A fortnight later the lethal temperature was found to be 61°. These temperatures cannot be taken as conclusive, and as the death-point was not increased by subsequent sub-culture, the lethal temperature remains unknown.

With regard to the cultural and other characters of the coccus, it measures 1μ, stains well and is Gram-positive. Upon agar, there forms a porcelain-white, raised and fat-glistening growth. Bouillon becomes turbid and a coherent sediment is produced;

indol is formed and nitrates are reduced to nitrites. On potato a moist transparent growth is slowly formed. Gelatine is slowly liquefied, and no gas is produced from glucose. In stab-culture, the gelatine is softened and the growth gravitates. It is not pathogenic to mice.

These characters show that it is only distinguishable from *Micrococcus pyogenes* γ *albus* (Rosenbach) by its being non-pathogenic to mice.

Postscript (*added 30th April, 1909*).—After this paper was read, a fresh specimen of coagulated milk was obtained. The micrococcus was isolated and its lethal temperature tested immediately after isolation. The infected milk exposed for 10 minutes at 62° was coagulated and at 63° was unaltered upon incubation.